LBNL Seeks Licensees for Highly Specific and Sensitive DNA Extraction Method

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By Ben Butkus

Lawrence Berkeley National Laboratory has made available for licensing a DNA extraction and isolation method that its inventors claim is more efficient, sensitive, and selective than current commercial DNA extraction kits.

In particular, the new technique may be especially valuable for downstream applications where the extraction of minute amounts of DNA plays a critical role, such as basic and applied biology research, forensics, biosecurity, and environmental testing, according to the technology's inventors.

"This is a general method to get DNA out from any kind of sample, but with higher sensitivity, we think, than current methods, and more … versatility in terms of product models," Youn-Hi Woo, a staff scientist at Berkeley Lab and one of the method's inventors, told *PCR Insider* this week.

"It has a very broad field of use — anywhere someone wants a very small amount of specific DNA from larger samples or a larger pool," she added.

According to the LBNL researchers, the most popular current commercial DNA extraction methods use detergents, paramagnetic particles, or membrane filters. Each of these methods works well for certain applications, but each also has drawbacks, such as non-specific DNA separation and contamination with salts or negatively charged polymers. In almost all cases, the various methods require that researchers perform extra time-consuming or laborious wash steps.

Furthermore, although paramagnetic particles eliminate many of the chemistry-related problems, they are difficult to employ using large sample volumes, meaning that researchers must first concentrate a sample down to microliter-scale volumes or less. This is particularly daunting with the larger-volume samples commonly found in environmental testing or forensics.

The new DNA extraction protocol, which the LBNL researchers described in a paper published earlier this year in *Analytical Biochemistry*, relies on the combination of the DNA-specific enzyme

methyltransferase, or DNA Mtase, and so-called "click" chemistry, which has the ability to irreversibly couple two molecules under mild conditions.

More specifically, DNA in a complex sample is selectively labeled using MTaqI, an Mtase derived from *Thermus aquaticus*, and with alkynyl-SAM, a cofactor molecule that supplies methyl groups that the MTaqI transfers to the DNA when it recognizes short nucleotide sequences.

Then, the mixture is applied to an azide-modified click chemistry surface in the presence of copper ions, where the selected DNA molecules become covalently bound. Standard or vigorous washing steps wash away any contaminants, leaving behind only the desired DNA molecules bound to the modified surface.

"One strength of this technology … is pulling out DNA by covalent bonds," Woo said. "This means it can't be pulled off easily. You can be pretty harsh in the washing steps to get rid of whatever the DNA was contaminated with. This gives you [more] freedom in what you do to purify your sample."

In their proof-of-concept *Analytical Biochemistry* paper, Woo and colleague Alexander Artyukhin of Lawrence Livermore National Laboratory demonstrated that their method could capture DNA at the femtogram level, or close to a single copy number, in a mixed sample. Comparatively, most currently available commercial DNA extraction kits show nanogram-level sensitivity.

In addition, the researchers demonstrated that they could use either modified silica beads or a planar surface to capture DNA.

"[We believe] that this surface modification can be applied to … magnetic or plastic beads, or plastic tubes, or even a glass surface," Woo said. "Or, if you are dealing with a very small amount of sample, we can use it in kind of a microfluidic kit setup. So there is just a diversity of formats we can develop."

Woo added that it would be particularly desirable to modify the inner surface of a standard Eppendorf tube, "so you can have your DNA isolated already in your tube, so you don't even have to transfer it from there for the next process, like downstream analysis."

Woo and Artyukhin also showed that their method works over a wide pH range and in the presence of varying concentrations of common salts; and used their method to isolate phage DNA from soil samples and to perform PCR on bead-bound isolated DNA molecules either added to a mixture or derived from bacterial cells whose membranes had been ruptured.

Woo said that while the method provides some sensitivity advantages over commercial kits, in terms of reducing workflow, it doesn't confer a significant advantage in cases of "simple genomic DNA extraction, like if you have tons of a [bacterial] sample."

However, where the technique really shines is in extracting small amounts of DNA from large volumes of mixed and/or dirty samples.

"For instance, if you have seawater, and you're looking for specific DNA, which will be something like one [molecule] per billion, then you [typically] have to somehow concentrate down this sea water into a workable sample size in the lab, then go through a lot of concentration or cleanup processes," Woo said.

"But we expect using this technology that you won't need to go through this concentration step," she added. "Let's say you have liters of your seawater — you can add your beads and

enzymes, and then pull out very small trace amounts of DNA without all these extra steps to purify and concentrate."

The researchers are already taking advantage of this feature in a collaboration with NASA's Jet Propulsion Laboratory. Specifically, scientists there are exploring the use of Woo's method to isolate and profile DNA from any potential bio-contaminants on spacecraft both before and after missions.

In addition, the Berkeley Lab project was originally funded by the Department of Homeland Security's bioforensics program, Woo said, adding that "forensics people are definitely interested." In general, she noted, the DNA extraction method is ideal for applied testing markets, but that it should also be a powerful tool in biomedical research workflows, such as purifying DNA for downstream PCR or sequencing analysis, or even for clinical applications.

Still, Woo said, the technology is at a very early stage, and the group continues to make modifications that could move the technology closer to commercial viability. For instance, some early collaborators have expressed an interest in being able to remove the DNA from the modified surfaces after isolation. "So right now we are trying to create a little [photocleavable] linker, so when the purification is done, we just [shine light] on it to cleave off the DNA."

In addition, "researchers don't want sheared DNA, so we want to try and keep the DNA as intact as it should be. So we're still working things out there," she said.

The technology is currently patent-pending, and Woo's group is seeking collaborators, particularly commercial entities, to license the new method and help develop products based on it.

Besides its collaboration with the NASA Jet Propulsion Laboratory, the Berkeley Lab is also currently working with the Joint Genome Institute and DHS Forensics Centers to evaluate and apply the method.

Ben Butkus is senior editor of GenomeWeb's premium content and the editor of *PCR Insider*. He covers technologies and trends in PCR, qPCR, nucleic acid amplification, and sample prep. E-mail him here or follow his GenomeWeb Twitter account at @PCRInsider.

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